United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program

SOP No.: MDP-MTH-05		Page 1 of 9
Title: Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX® System		
Revision: Original	Replaces: NA	Effective: 04/15/04

1. Purpose:

To provide standard procedures for use of the BAX® system for detection of *Escherichia coli (E. coli)* O157:H7 in fresh produce by all laboratories participating in the USDA/AMS Microbiological Data Program (MDP).

2. Scope:

This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program.

3. Principle:

The BAX® system is a DNA-based screening method for detecting pathogens in food and environmental samples developed by DuPont Qualicon. The sensitivity and the accuracy in detection are a result of the use of polymerase chain reaction (PCR) to amplify specific DNA fragments.

4. Outline of Procedures:

5. References:

5.1 SOP MDP-LABOP-02, Sample Receipt and Elution Procedure

5.2 SOP MDP-MTH-06 *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Confirmation

5.3 SOP MDP-DATA-01 Microbiological Record Keeping and Results Reporting

5.4 Peter Feng and S. D. Weagant. BAM Chapter 4A. Diarrheagenic *Escherichia coli*. http://www.cfsan.fda.gov/~ebam/bam-4a.html (last accessed 2/23/04)

5.5 BAX System, User Guide and Protocol Summary, DuPont Qualicon.

6. Specific Procedures:

6.1 Equipment and Materials

6.1.1 Incubators, 2 (35 ± 2ºC and 37 ± 0.5ºC)

6.1.2 Heating blocks, 2 (37 ± 0.5ºC and 95 ± 1°C)

6.1.3 Cooling block

Note: *Ensure that cooling block is refrigerated overnight prior to beginning assays.*

6.1.4 Dupont Qualicon BAX instrument

6.1.5 BAX system PCR assay kit for *E. coli* O157:H7 (order # 17710611)

6.1.6 BAX System User Guide

6.1.7 Sterile pipets (1 mL and 10 mL) and pipet aids

6.1.8 Micropipettors capable of delivering 5-200 µL with disposable sterile filtered micropipette tips

6.1.9 Lysis tubes, caps, optical caps, and capping tools for BAX

6.1.10 Sterile tubes

6.1.11 Lab coats, powder-free gloves and eye protection glasses

6.2 Media and Reagents

6.2.1 Modified EC Broth (mEC) + novobiocin

6.2.1.1 If pre-made mEC medium and novobiocin (in powder or liquid form) are used, follow the instructions from the supplier.

6.2.1.2 If preparing medium, use ingredients listed in table below:

Tryptone	20.0 g
Bile salts #3	1.12 g
Lactose	5.0 g

K_2HPO_4	4.0 g
KH_2PO_4	1.5 g
NaCl	5.0 g
Water	1.0 L

Adjust the pH to 6.9 ± 0.1 with 1N HCl before autoclaving. Autoclave the broth at 121°C for 15 minutes and cool. Add 5 mL of a filter sterilized aqueous solution of 4 mg/mL novobiocin to a final concentration of 20 µg/mL.

6.2.2 Non-specific rich broth such as Lactose Broth and Brain Heart Infusion (BHI)

6.2.3 *E. coli* O157:H7 strain ATCC 43890-GFP as positive culture control grown overnight in a non-specific rich broth and subcultured the next morning in a non-specific broth to a 0.5 McFarland standard (approximately 4-5 hours).

6.2.4 *E. coli* as negative culture control grown overnight in a non-specific rich broth and subcultured the next morning in a non-specific broth to a 0.5 McFarland standard (approximately 4-5 hours).

6.3 Controls

6.3.1 Use the controls for each batch of samples and carry forward through the remaining test steps and the BAM culture confirmation steps if a confirmation is needed on a sample. If the positive controls fail to yield a satisfactory result or there is any question about the performance of the testing because of the control results, refer to SOP MDP-QA-01.

6.3.2 List of Controls

6.3.2.1 Negative Media Control: 25 mL BPW plus 0.1% Tween 80 (See SOP MDP-LABOP-02) to 225 mL sterile mEC + novobiocin (mEC+n) broth

6.3.2.2 Negative Culture Control: 1 mL *E. coli* culture in 225 mL sterile mEC broth without novobiocin

6.3.2.3 Positive Culture Control: 1 mL *E. coli* O157:H7 strain ATCC 43890-GFP culture in 225 mL sterile mEC+n broth

6.3.2.4 Positive produce culture control: After eluate has been inoculated into test cultures and at least 15 mL eluate has been saved for possible re-testing, choose a single produce sample at random and add 1 mL of the *E. coli* O157:H7 positive control culture. Gently mix by hand – do not use shaker;

1 mL of the produce control eluate is inoculated into 225 mL of sterile mEC+n broth.

6.4 BAX Analysis:

6.4.1 Pre-enrichment and Preparation of Samples and Controls

6.4.1.1 Remove a 25 mL aliquot of each sample (produce wash eluate) and place into a suitable sterile container. Add 225 mL mEC+n to each of the containers. Incubate samples 14-24 hours at 35 ± 2ºC.

6.4.1.2 Refrigerate the unused mEC+n broth culture until the BAX analysis is done. This sample may be required for isolating *E. coli* O157 cells from BAX positive samples by Immunomagnetic Separation (IMS) system.

6.4.1.3 Turn on the BAX and then the associated computer. Launch the BAX application. Perform verification if needed (every two weeks – see BAX User Guide, Chapter 3, for instructions).

6.4.1.4 Create a Rack File. This may be performed manually or by using the Rack Wizard. Define all wells for analysis, including controls. (For step-by-step directions, see the BAX User Guide, Chapter 2). The cycler should be ready when samples have been prepared.

6.4.2 Pooling, Regrowth, and Lysis

6.4.2.1 Thoroughly suspend mEC+n broth pre-enrichment culture. To pool three samples, add a 10 µl aliquot of each of the three pre-enriched samples to a 500 µL tube of BHI broth. Incubate for 3 hours at 37 ± 0.5°C (alternatively, heating blocks may be used).

Note: To minimize cross contamination arrange the samples, positive controls, and negative controls in a manner to avoid contamination by aerosols. Physical separation during preparation is recommended. Careful pipetting is essential.

6.4.2.2 Prepare lysis tubes. From the Qualicon supplied kit, add 150 µL of protease to one 12 mL bottle of lysis buffer. If a smaller volume is needed, use 12.5 µL protease to 1 mL lysis buffer. Prepared lysis reagent may be stored up to two weeks at 2-8ºC.

6.4.2.3 Transfer 200 µL of Lysis Reagent to each lysis tube.

6.4.2.4 Transfer 5 µl BHI-grown sample to lysis tubes using the micropipettor. Cap with lysis tube caps.

6.4.2.5 Heat tubes for 20 min. in 37 ± 0.5 °C heating block.

6.4.2.6 Move tubes to 95 ± 1°C heating block for 10 minutes.

6.4.2.7 Place lysis tubes in cooling block for 5 minutes. Complete use of all cooling blocks within 30 minutes of removal from refrigerator.

6.5 Use of BAX Instrument

6.5.2 Begin heating cycler by clicking the "Run Full Process" icon on the BAX computer. Place a PCR tube holder into the PCR cooling block. Place one PCR tube per sample and one per control into the holder, according to Rack File.

6.5.3 Remove PCR caps using decapping tool and discard. Remove cap from lysis tube using decapping tool.

6.5.4 Transfer 50 µL of lysed sample from lysis tube into corresponding PCR tube. Repeat for all samples and controls. Cap tubes with optical caps using the capping tool. Keep samples in cooling block until cycler is ready for loading.

6.5.5 When prompted by instrument, load rack. Follow prompts to begin cycling process.

6.5.6 After PCR and detection processes are complete, remove samples and follow prompts to display results.

6.6 Reporting

Data shall be reported according to SOP MDP-DATA-01.

6.7 Confirmation

6.7.1 When a pooled sample is positive via BAX, the test shall be repeated on the three individual samples. From each individual refrigerated mEC+n broth sample, add a 10 µL aliquot to a 500 µL tube of BHI broth. Incubate for 3 hours at 37 ± 0.5°C. Refrigerate the unused mEC+n broth until the BAX analysis is complete. Follow this SOP from Section 6.4.2.2.

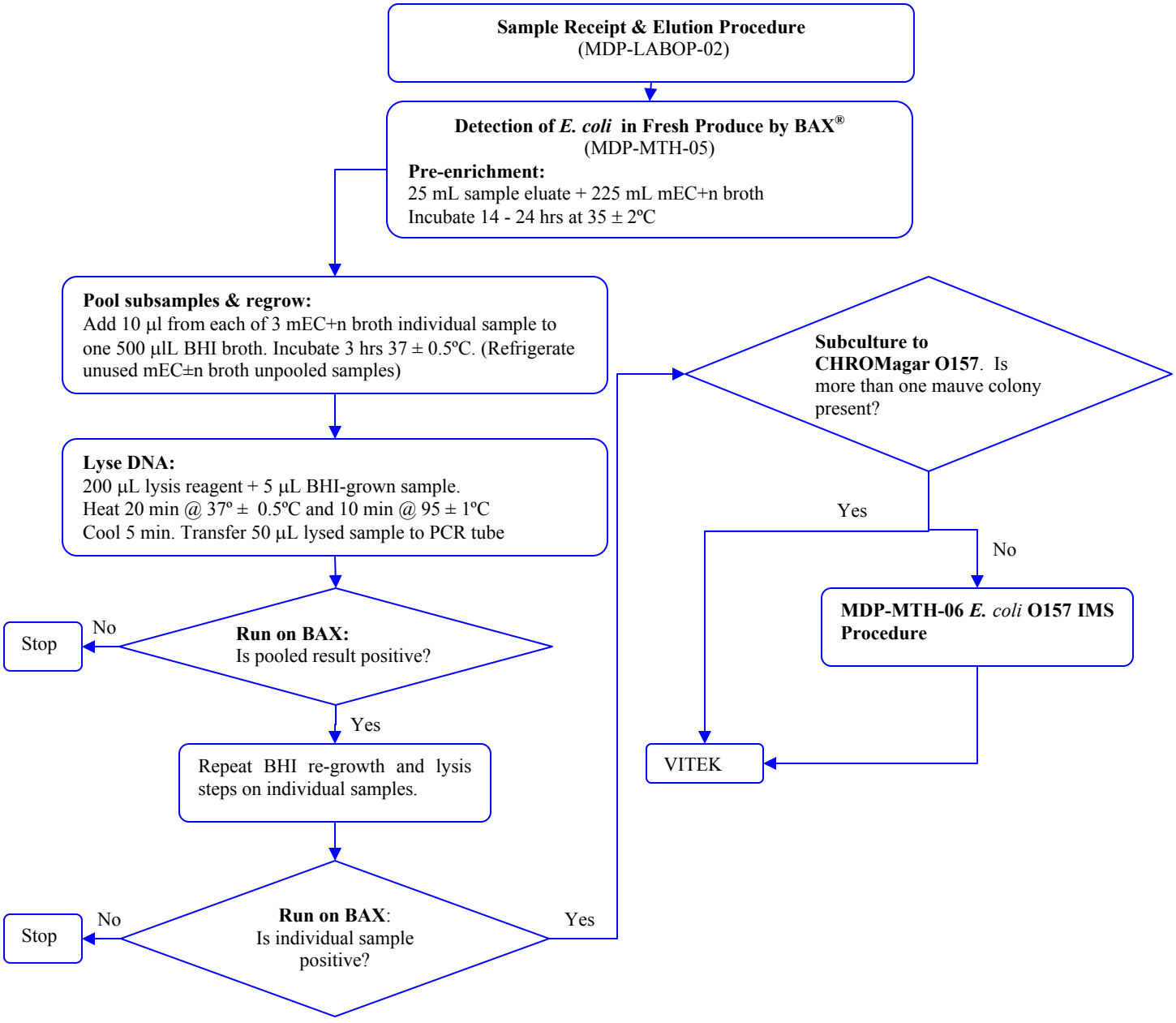
6.7.2 Inoculate the unpooled BAX-positive sample on CHROMagar O157 plate(s) using a sterile swab by spreading sample over half of the plate. Using a sterile loop, streak back and forth between the swabbed and unstreaked areas several times. Incubate the plate at 35 ± 2ºC for 18-24 hours.

6.7.3 Observe for *E. coli* O157 colonies which appear mauve. To ensure the presence of *E. coli* O157, confirm more than one colony using VITEK. Report results and prepare for shipment according to MDP-MTH-06.

6.7.4 If no mauve colonies are found, proceed to MDP-MTH-06. Confirmation may be performed on the same or following day.

7. Safety:

E. coli O157:H7 is a human pathogen and is shown to cause disease with a low infectious dose. The laboratory personnel must follow CDC guidelines for working with Class II pathogens. Use of lab coats, gloves and eye protection is mandatory. A Class II biosafety laminar flow hood (cabinet) is recommended.


Sample Receipt & Elution Procedure
(MDP-LABOP-02)
Detection of E. coli in Fresh Produce by BAX®
(MDP-MTH-05)
Pre-enrichment:
25 mL sample eluate + 225 mL mEC+n broth
Incubate 14 - 24 hrs at 35 ± 2ºC
Pool subsamples & regrow:
Add 10 µl from each of 3 mEC+n broth individual sample to one 500 µlL BHI broth. Incubate 3 hrs 37 ± 0.5ºC. (Refrigerate unused mEC±n broth unpooled samples)
Lyse DNA:
200 µL lysis reagent + 5 µL BHI-grown sample.
Heat 20 min @ 37º ± 0.5ºC and 10 min @ 95 ± 1ºC
Cool 5 min. Transfer 50 µL lysed sample to PCR tube
Run on BAX:
Is pooled result positive?
No
Stop
Yes
Repeat BHI re-growth and lysis steps on individual samples.
Run on BAX:
Is individual sample positive?
No
Stop
Yes
Subculture to CHROMagar O157. Is more than one mauve colony present?
Yes
No
MDP-MTH-06 E. coli O157 IMS Procedure
VITEK

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- Established procedures for testing of MDP samples for *E. coli* O157:H7 using the BAX® instrument.